Conclusions: Treatment with GTN increased SCB thickness and density during OA. NO inhibits osteoclastic bone resorption *in vitro*, and NO-donors have been shown to increase bone density elderly women. High BMD has been associated with OA, whilst subchondral bone sclerosis is a histological hallmark of OA and is suspected by some to be central to its pathogenesis. The results of this study suggest that NO may be an important mediator of the SCB changes seen in OA, and provides evidence that clinical use of NO donor compounds, such as GTN, may contribute to disease progression in OA.

PB37

BIOLOGICAL ACTION OF HYALURONAN ON HUMAN ARTICULAR CHONDROCYTES: THE IMPORTANCE OF MOLECULAR WEIGHT

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Introduction: Hyaluronan.(HA) is a glycosaminoglycan (GAG) which is present in synovial fluid and the extracellular cartilage matrix (ECM). HA is viscoelastic and concentration of HA in OA synovial fluid (SF) is lower than normal SF. Original use of intra-articular (IA) HA in OA was to increase the viscosity of SF. Clinical effects with IA HA prolong the life of HA in the joint. These findings can not be explained only by the biomechanical properties.

Aims: To analyze the biological effects of HA on NO and PGE2 synthesis, and on chondrocyte apoptosis. To study the importance of the molecular weight of HA on these biological effects.

Material and Methods: Cells were isolated from cartilage obtained from joint replacement in OA patients. Chondrocytes (75 000 cell/well) were cultured for 24h to detect PGE2 or 48 hours to measure NO, afterwards they were pre-incubated with HA and stimulated with IL-1 5 ng/ml. We have used an HA of low molecular weight (HALMW): Hyalgan® (500-730 kDa, Bioiberica S.A.) and another HA of high molecular weight (HAHMW): Synvisc® (6,000 kDa, Biomatrix Inc), at the following concentrations: 10 10 λg/ml; 50 λg/ml; 100 λg/ml; 150 λg/ml and 200 µg/ml. NO was detected by Greiss reaction and PGE2 was quantified by a commercial EIA (Amersham) in supernatant. Apoptosis was induced by an NO donor and the effect of HA on apoptosis was quantified by flow cytometry.

Results: Both HAs studied do not have any effect on basal production of NO or PGE2. However, HALMW at 200 λg/ml reduces by 70% the NO synthesis and by 45% the synthesis of PGE2 induced by IL-1. HAHMW does not modify the levels of NO or PGE2 induced by IL-1. Both HAs at 200 mg/ml decrease the apoptosis induced by NO (HALMW by 40% and HAHMW by 3 6%).

Conclusion: In this study, the analysis in chondrocyte of two HAs has evidenced that different HAs have different effects. Therefore, we can state that molecular weight influences some of the biological effects that this molecule has on human articular chondrocytes.

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PB38

EFFECTS OF ELASTOVISCOUS SUBSTANCES ON THE MECHANOSENSITIVITY OF ARTICULAR PAIN RECEPTORS

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Aim: Elastoviscous hyaluronan solutions reduce articular pain in humans and impulse activity of nociceptors ('pain receptors') in experimental animals. The aim of this work was to compare the effects of commercial viscoelastic substances with different molecular weights on the discharges of joint nociceptors evoked by movement of the inflamed rat knee joint.

Methods: Electrical activity of single fibers innervating the knee joint was recorded from fine filaments of the median articular nerve of anesthetized rats. Knee joint inflammation was produced by intraarticular injection of kaolin and carrageenan. Mechanical stimulation consisted in controlled rotations of the knee joint within and outside its normal working range, for 10 s every 5 mm. Solutions of sodium hyaluronan of different molecular weights (MW) (<1,000,000 and <2,000,000) and of the high molecular weight (v 6,000,000) hyaluronan derivative hylan G-F 20 were injected into the joint cavity (0.05 m1). Impulse discharges evoked by the movements were compared before and after injection.

Results: Hylan G-F 20 significantly reduced in the course of 1 hour the number of nerve impulses evoked by movements to about 60 % of the control frequency. MW <2,000,000 hyaluronan solutions had a slightly faster (around 30 min) and weaker, but still significant effect (reduction to 75% of the control impulse frequency); MW <1,000,000 hyaluronan solutions did not decrease significantly the mean impulse frequency of movement-evoked discharges in pain nerve fibers.

Conclusions: It has been proposed that hyaluronan-derived elastoviscous substances used for the treatment of articular pain act as a viscoelastic filter, reducing the transmission of stretch to pain nerve terminals in the joint. High molecular weight hylan solutions were more effective than lower molecular weight hyaluronan solutions in reducing nerve discharges of pain fibers. Thus, it is to be expected that high MW hylan solutions will have more pronounced analgesic effects on human joint pain than hyaluronan solutions of lower MW.

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PB39

HUMAN SERUM IS SUPERIOR TO FETAL CALF SERUM FOR ISOLATING AND ESTABLISHING MONOLAYERS OF HUMAN ARTICULAR CHONDROCYTES

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The presence of serum is indispensable for growing many cell types culture. Fetal calf serum (FCS) is commonly used for establishing monolayers of articular chondrocytes (AC) and is considered as the best source of factors promoting cell growth. The development of techniques of chondrocytes' grafting on damaged articular surfaces has stressed in recent years a need for the use of human serum (HS) for preparing and cultivating human AC.